THE MAIN FACTS IN REGARD TO THE CELLULAR BASIS OF HEREDITY.

(Contributions from the Zoological Laboratory of the University of Texas, No. 56.)

BY THOMAS H. MONTGOMERY, JR.

(*Read January 15, 1904.*)

I.

Under heredity we understand the transference to the offspring of qualities of the parent or parents. The interpretation of the phenomena involved constitutes one of the broadest problems in the field of Biology, and has for centuries been the theme of eager discussion. Yet only in the past forty years has there come out any positive knowledge upon the subject, except the making known of certain cases of parthenogenesis and of the occasional difference of reciprocal crosses.

There are obviously two methods of determining the facts of heredity. First, by the intercrossing of different varieties or species, and the determination of the relative influences of the parents upon the offspring. The first fundamental work in this line was done by Mendel in 1865 (*Versuche über Pflanzenhybriden*), who determined a large series of facts for the plant genus *Pisum*, and from the data established a mathematical law for this genus as to the inheritance of parental qualities by the hybrids. This memoir, only some three years ago resurrected from its long obscurity, is to-day occupying the attention it deserves, and has stimulated much work along the same line. De Vries' magnificent work, *Die Mutationstheorie*, demands as well recognition in this respect. But it is clear that such experimental intercrossing, in so far as only the end results of the crosses are considered, can do no more than state the degrees of resemblance of the offspring to the parents, and decide the questions as to the fertility of the hybrids. Important and necessary as it is, it does not go to the root of the matter, and cannot present any empirical analysis of the underlying factors.

For an understanding of these we must turn to the second method, to the examination and interpretation of the intimate structural and growth phenomena of the germ cells themselves, that is, to the

cellular basis. All explanations must remain purely hypothetical until this is done. And here I would call attention, as briefly and concisely as possible, to certain positive results that have been won in the study of the germ cells, and disregard the many fascinating but purely hypothetical views as to the process of heredity.

II.

The statement of the problem must be a very broad one. The fertilized egg gradually cleaves into many cells. These progressively arrange themselves into tissues, and these form organs. By continuing cell division, by change of position and infolding of cells, and particularly by a differentiation of the cells as the development proceeds, the adult organism eventuates. Then from the body of this adult comes an egg, and it repeats the whole involved process. Here are two great fundamental problems: the one, why the offspring resembles the parent? the other, what are the factors of differentiation? On the answer to these problems depends to great extent the explanation of how variations arise and how they are promulgated, that is, the explanation of descent with modification, broadly called evolution. The very subsidiary question of the determination of sex is necessarily also connected with these problems. And all of these questions are inseparable from the one: How far is the adult preformed or prelocalized in the germ cells?

What interests us immediately are the two points: First, has there been empirically determined a particular cellular substance, most intimately connected with the transmission of hereditary growth energies? And second, if such a substance is known, does its behavior during the process of development of the embryo throw any light upon the processes of heredity?

III.

To make the following argument clear, we must call to mind the structure of the mature germ cells and the process of cell division.

The maternal germ cell, the ovum, appears much like any large, unspecialized cell. We distinguish in it a central rounded body, the nucleus, with its surrounding cytoplasm. In the cytoplasm there is a living substance, the protoplasm proper, and various deutoplasmic substances, such as yolk, which serve mainly for the nourishment of the cell. The nucleus is more complex. Travers-

ing the thinly fluid nuclear sap, which fills it, is a delicate network or meshwork of linin threads, and supported upon or imbedded in them masses of a substance called chromatin. In the nuclear sap may be suspended also one or many large rounded bodies, the nucleoli, and numerous minute lanthanin granules. The whole is enclosed by a nuclear membrane.

The paternal germ cell, the spermatozoon, has a very different appearance, and in volume is exceedingly smaller than the ovum. In the case of the sea-urchin, Wilson (*The Cell in Development and Inheritance*) has computed it to be about one half-millionth the volume of the egg, and the difference is many times greater than this in the case of the bird. The history of its formation shows it to be a highly specialized cell with regard to its cytoplasm, which is generally modified to form a locomotory flagellum. But its amount of chromatin is the same as that in the egg cell, though contained in a very condensed form (composing the head of the spermatozoon). At the junction of the flagellum and head there is frequently found a mid-body, a metamorphosed centrosome. Thus there is a division of labor between the two germ cells: the ovum is large to provide the necessary cytoplasm and nourishment for the embryo; the spermatozoon minute and motile in order to reach the ovum.

All cell reproduction is by division of the cell, and the mode of division, which differs very notably from a mere constriction into two, may be briefly recalled. The nucleus of the cell increases in volume, and its scattered chromatin masses group themselves evenly along the linin threads, so that eventually the chromatin seems to be arranged in the form of a long, continuous loop. In the cytoplasm at one side of the nucleus appears a minute body, the centrosome. This divides into two centrosomes, and they wander apart from each other, each through an angle of 90°, to opposite sides of the nucleus. These centrosomes are the dynamic centres of the cell division and exert an influence upon the surrounding cytoplasm, as shown by systems (asters) of cytoplasmic rays converging upon them. Within the nucleus, meanwhile, the chromatin loop has become split through its entire length by an exact halving of each of its larger chromatin masses, and has also broken transversely into a fixed number of segments, the chromosomes, which now are connected together only by linin threads. Then the nuclear membrane dissolves away and a dicentric figure appears

with a centrosome, the centre of an aster, at each pole, the chromosomes grouped together in a plane midway between the poles and with the long axis of each chromosome coinciding with this plane. Then begins the separation from each other of the halves of each longitudinally split chromosome and to opposite poles, probably due to the contraction of linin fibres that connect the chromosomes with the centrosomes. Their separated halves come to lie in two groups, one near each centrosome. Finally, each centrosome loses its influence upon the cytoplasm, the radiations around it disappear, each group of chromosomes forms again a rounded nucleus, the cell body constricts between them to form two cells, and as a result there are two cells each with its own nucleus. The remarkable accomplishment is an exactly equal distribution of the chromatin mass to the daughter cells by a very complex mechanical process.

IV.

Now is there any particular one of these structures that can be determined as the bearer of hereditary qualities? No one has advocated that it might be a centrosome, and, indeed, there is no reason for considering a centrosome to be any other than a dynamic centre. Such a substance must then be in either the cytoplasm or the nucleus.

The earlier views were that this particular substance was located in the cytoplasm (Lankester, 1877; Whitman, 1878; Flemming, 1882; Van Beneden, 1883). But these were hypothetical assumptions and employed not so much to show a special hereditary substance, as rather to explain the progressive specialization of the cleavage cells. Hereditary traits cannot, moreover, be transmitted by the cytoplasm of the spermatozoon, for in some cases (Echinoderms) the whole cytoplasmic flagellum of the spermatozoon is left outside the egg, and only the head and midbody of the spermatozoon penetrate the egg in fertilization. There is also the decisive experiment of Boveri, to which we shall recur, showing that the cytoplasm of the egg cell also does not transmit hereditary traits.

Accordingly the hereditary substance must have its seat in the nucleus, and there is now practically positive evidence that such a germ plasm is the chromatin. The main reasons are as follows:

(1) The exact distribution of the chromatin in cell division, so that each daughter cell receives just half the amount of chromatin

of the mother cell. The longitudinal splitting of the chromosomes is an autonomous act, whereby each small chromatin mass composing the chromosome (though not the smallest visible granules or microsomes) divides exactly into halves, and the whole complex series of changes leading to the dicentric division figure seem to have been evolved simply to effect the equal distribution of the daughter chromosomes to the daughter cells. Whether the cytoplasm divides equally or unequally, the chromatin is always divided and distributed equally. This fact alone has seemed sufficient to most workers to mark the chromatin as the hereditary substance.

(2) The fact that the chromosomes, the accumulations of chromatin during cell division, are fixed in number for all the cell generations of a species. And the strong probability, amounting almost to a fact, that the chromosomes preserve their individual continuity from generation to generation, notwithstanding their great chemical and structural changes during the rest stage of the cell.

(3) The fact that the spermatozoon, in most respects the very antithesis of the ovum, on entering the egg in fertilization brings in just the same amount of chromatin as that contained in the egg. Not only is this so, but Van Beneden demonstrated as long ago as 1883 (*Recherches sur la maturation de l'œuf*) that the spermatozoon brings into the egg just as many chromosomes as are contained in the latter. Since we know that the two parents have an approximately equal influence upon the offspring, and since the chromatin is a substance contributed in equal amount by the two germ cells, it is logical to conclude that this substance is the seat of the hereditary growth energies.

(4) The fact that, despite considerable differences in other respects in their cell divisions, animals and the higher plants show essentially the same behavior of the chromosomes.

(5) The experiment, first made by Boveri, 1895 (*Ueber die Befruchtungs- und Entwickelungsfähigkeit kernloser Seeigel-Eier*), of fertilizing with a spermatozoon the cytoplasm of an egg cell deprived of its nucleus. Such a fertilized egg fragment develops, but shows purely parental characters, probably because all maternal chromatin had been eliminated. And two recent papers by Boveri (*Ueber mehrpolige Mitosen*, etc., 1902; *Ueber den Einfluss der Samenzelle auf die Larvencharaktere*, 1903) have shown, with

their keen critical analysis of the experiments, that the chromatin alone can be considered the bearer of the hereditary traits.

From all these results it is concluded that the chromatin is the seat of the hereditary growth energies.[1]

And from another point of view this is rendered probable. The microchemical study of the cell has shown that the chromatin is the most active substance concerned in cellular metabolism; and experimental work, particularly that of Verworn, shows that a cell deprived of its nucleus, and hence of its chromatin, is unable to build up new substances. The chromatin accordingly, as it is transmitted from generation to generation, carries with it certain definite metabolic energies characteristic of the species. And from this view there is good reason to consider the idea of Delage (*La structure du protoplasma et les theories sur l'Hérédité*, 1895) to be in the main correct, namely, that the offspring is like the parent because it has similar metabolic energies.

V.

There is another series of facts known about the behavior of the chromatin, the hereditary substance, in the germ cells, and a few of them will be touched upon. Oscar Hertwig showed, in 1875 (*Beiträge zur Kenntniss der Bildung, etc., des tierischen Eies*), that the fertilized egg cell contains two nuclei, one belonging to the egg cell itself and one introduced by the spermatozoon. Then Van Beneden (*l. c.*) demonstrated that the spermatozoon brings in just

[1] It has been argued by an English writer whose name escapes me, as does the title and date of his paper, that the linin is the hereditary substance. Active chromatin is never disassociated from linin, but there is always a substratum of linin in each chromosome, and in the rest stage the chromatin is always supported upon linin strands. Hence it was argued that the linin is likewise equally distributed in cell division. This is a good point, but there is a strong objection to it. When the daughter chromosomes separate, in the anaphase, the linin becomes pulled out between every two corresponding chromosomes as a connective fibre, and in the reconstruction of the daughter nuclei the greater portion of such a fibre is not taken up again into the nuclei. And this fact cannot be used in favor of the intracellular pangenesis theory of de Vries, whereby pangenes are hypothetically supposed to wander out of the nucleus and so determine the differentiation of the cleavage cells, for the connective fibres appear to behave alike in all cell divisions. Thus of the two constituents of the chromosomes, at each cell division some of the linin becomes displaced into the cytoplasm, but all the chromatin passes into the nucleus.

as many chromosomes, and that their mass is the same, as those contained in the egg. Further, it is proved that in normal fertilization only one spermatozoon enters the egg, and that when more than one enters the development is abnormal. The proof that both egg chromosomes and sperm chromosomes have an approximately equal rôle in determining the growth of the embryo has been shown by Boveri (*l. c.*) by crossing different species of sea-urchins, and by analyzing the results of fertilizing an egg with two or more spermatozoa.

Now each act of fertilization would necessarily double the normal number of chromosomes, since the spermatozoon introduces as many as are already present in the egg, were there not some process to obviate this. There is such a process, and it is known as the "reduction in number of the chromosomes." The last two divisions of the germ cells, preceding the act of fertilization and preparing them for it, are known as the maturation divisions; and it has been known for some fifteen years past that in these divisions each germ cell has only one-half the normal number of chromosomes. It is also proven that the ripe egg cell, as well as the ripe spermatozoon, has only one-half the number of chromosomes characteristic of the species. It is further known (since the work of Henking and of O. Hertwig, in 1890) that the processes involved in producing this result are essentially the same in both germ cells. Accordingly, by this preliminary halving of the number in each germ cell before fertilization, the germ cells on conjugation each contribute only one-half the normal number, with the result that the normal number is restored. But this preliminary reduction in number has a broader meaning than this.

Before the first maturation division of the germ cell is accomplished there takes place a pairing of the chromosomes, so that instead of, *e.g.*, four single (univalent) chromosomes there are two double (bivalent) ones (Montgomery, *Spermatogenesis of Peripatus*, 1899). These become so arranged that one of the two maturation divisions results in separating chromosomes that are split longitudinally, just as in any other cell division; but the other maturation division removes entire chromosomes from each other by separating the two chromosomes of each pair, and thereby reduces the number of the chromosomes to one-half. That is definitely known for certain species.

But how account for the preliminary pairing of the chromosomes? It is apparent that each spermatozoon may be called paternal, but not male, and each egg cell maternal, but not female, for the following reason: We have seen that each organism formed by fertilization has a fixed number of chromosomes, half of which were derived from the spermatozoon and half from the egg cell. The germ cells that develop within that organism, be they spermatozoa or egg cells, accordingly have an equal number of chromosomes from each parent. Therefore, the spermatozoon contains maternal as well as paternal chromosomes, and the egg cell paternal as well as maternal chromosomes. And, therefore, each germ cell has in equal measure the hereditary substance of both its parents.

Now the process of pairing of the chromosomes, which we found to be an initial step to their reduction in number, has been proved to be a pairing of paternal with maternal chromosomes (Montgomery, *A Study of the Chromosomes of the Germ Cells of the Metazoa*, 1901). In a particular generation of the sperm cell it was demonstrated (and not merely "surmised," as stated by another worker) that paternal chromosomes pair with maternal ones, forming thus double rods instead of single ones; it is probable, but not yet demonstrated, that likewise in each egg cell, of the corresponding generation, paternal chromosomes pair with maternal. Thus in the reduction division, which displaces the two elements of a pair, a maternal chromosome separates from a paternal in each pair, but not so that all the paternal chromosomes pass into one cell and all the maternal into another.

These facts which we have learned about the chromatim lead to a conclusion that for its probability approaches a fact. That is, that the different chromosomes in a germ cell have each their particular values. Roux (*Ueber die Bedentung der Kerntheilungsfiguren*, 1883) was the first to postulate that the chromatin cannot be hereditarily the same throughout the length of a chromosome, for otherwise its equal longitudinal splitting would be without meaning. In other words, each particular portion of a chromosome would represent a particular hereditary value. Not only is this probable, but it is also probable that one chromosome has hereditary values not found in the others. For we have seen that each germ cell has a set of maternal and a set of paternal chromosomes, and that in a particular generation those of the one set pair with those

of the other (Montgomery, *l. c.;* Sutton, *The Chromosomes in Heredity*, 1903). The two that pair are of corresponding volume (as brought out especially by Sutton), and sometimes of corresponding form (Montgomery, in a paper now in press). Because they are thus similar in volume and form, it is at least possible that they are similar in hereditary value. So Sutton has ably argued that when the two of a pair, a maternal and paternal chromosome of corresponding volume, separate from each other in the reduction division, chromosomes of like hereditary quality become separated into separate cells, so that no mature germ cell shall contain before fertilization two chromosomes having similar hereditary values. And this is the best reason yet given in explanation of the peculiar reduction division.

VI.

Finally, we may ask how far these facts agree with the germplasm theory of Weismann.

Some eighteen years ago, Carnoy (*La cytodiérèse chez les arthropodes*, 1885) showed, and he was the first to do so, that two kinds of cell division occur, namely, a transverse splitting of the chromosomes and a longitudinal splitting. That transverse splittings of chromosomes should occur was directly opposite to the prevalent view of the time, to the effect that only longitudinal divisions take place. Carnoy was far ahead of his day, and while this most important memoir of his then and for years afterwards met with only rather scornful criticism, we must now grant him his proper place as the discoverer of the reduction divisions.

Weismann, in 1887 (*Ueber die Zahl der Richtungskörper .und ueber ihre Bedeutung für die Vererbung*), prophesied, clearly without knowledge of Carnoy's work, and in conformity with the ideas of Roux (1883, *l. c.*), that in addition to the longitudinal splitting of the chromosomes, the "hereditary equal division," there would be found to occur in certain generations of the germ cells a "hereditary unequal division," either by a transverse division of the chromosomes or by a separation of entire chromosomes from each other. A number of the students of the maturation phenomena of the germ cells have empirically demonstrated this. Weismann's reduction division is the one where entire chromosomes become separated from each other. Equally, confirmation has been brought of another of his cardinal postulates, the con-

tinuity of the germ plasm. To be sure it is known that the germ plasm, the chromatin, is not an eternally unchangeable substance, as Weismann at first postulated. But the chromatin persists from generation to generation; the continuity of the germ plasm is what to-day is being termed the continuity of the chromosomes, and these continue from generation to generation, maintaining their individuality, just as much as a particular cell of one generation may be said to be represented by a particular cell of another.

Only some half dozen years ago, in the course of the conflict over the germ-plasm theory of Weismann, no workers upheld the occurrence of the reduction division except the school at Freiburg and one or two others. There even appeared a paper, presuming to be decisive, entitled "The Facts of Chromosome Reduction *versus* the Postulates of Weismann" (J. E. S. Moore, 1897). Since that time there has been much new research and by the comparative method, perhaps the safest of all methods, and the mass of evidence is now strongly corroborative of Weismann's two cardinal postulates. So to-day Weismann can point to the actual confirmation of the fundamental portion of his germ-plasm theory.

Stated Meeting, February 5, 1904.

President Smith in the Chair.

The following papers were read:

"The Babylonian and Hebrew Accounts of the Creation, in the Light of Recent Criticism," by Prof. Morris Jastrow, Jr.

"The Miocene Diabase of the Santa Cruz Mountains in San Mateo County, California," by H. L. Haehl and Ralph Arnold, communicated by Prof. J. C. Branner.